DURATION OF THE REACTION OF THE MEDULLA OSSIUM TO ACCLIMATIZATION TO HYPOXIA

N.P. Blagovestova, Ye.V. Loginova, Ye.Ye. Simonov and M.M. Fomenko

ABSTRACT: In the high-altitude and pressure-chamber experiments we studied the duration of the hemopoietic reaction in rats under various schedules of acclimatization to hypoxia. We judged the hemopoietic condition based on the change in the rate of proliferation of the medulla osseous elements, evaluated using the statmokinetic indices and the variation curves of the amount of DNA in the cellular nuclei. The amount of DNA was studied as a sign of the intensity of the synthetic processes which are the basis of preparing the cell for division. These investigations show that prolonged single and repeated residence of animals under conditions of lowered barometric pressure, corresponding to "altitudes" of 2000-7000 m above sea level, produce a significant increase in the amount of DNA-synthesized cells and the mitotic activity of the erythroblast elements which are noted for a period of 3 - 5 weeks following cessation of the experiments. The increase in the rate of proliferation of the cells of the series did not depend on the schedule of acclimatization and was observed even in such cases when the picture of the blood which is typical for hypoxic conditions was not evolved. The mitotic activity of the myeloid cells in the experimental rats, on the contrary, was suppressed. The changes in proliferative activity of the myeloidal cells permit us to trace not only the effect of the different schedules of acclimatization but the possibility of cumulation with repeated effects of hypoxia on the organism.

Numerous investigations by Russian and foreign authors have /198
shown the possibility of a temporary increase in tolerance to hypoxic effects under the influence of the proper training, producing a given "adjustment" of the adaptation mechanisms. Among these latter apparently of no little value is the duration of the "mobilization" readiness of a system as the hemopoietic system. At the present time we can find in the literature indications that traces of such readiness in the organs of the blood system are re-

tained for a significant period of time [3,5]. Unfortunately, such information is still quite small. Moreover, it does not perfectly reflect the influence of the training schedule on the duration of retention of the trace reactions in the hemopoietic tissues. This problem is of definite theoretical and practical interest since trace reactions in the medulle ossium may have substantial value for the cumulation of adaptation changes with repeated hypoxic effects. This fact caused us to undertake a special investigation, directed mainly to finding trace reactions in the medulla ossiu, after carrying out different types of acclimatization to hypoxia. For this purpose we set up several series of experiments with prolonged single and repeated residence of animals under conditions of a lowered barometric pressure, corresponding to altitudes of 2000-7000 m. We judged the hemopoietic condition for different periods of time after acclimatization from the change in the rate of proliferation of the medulla osseous elements, which in turn were evaluated from data on determining the statmokinetic indices and in a number of instances by comparative analysis of the variation curves of the amount of DNA in the nuclei of the individual cells of the medulla ossium.

METHOD

The experiments were set up on white rats (males) weighing 130 - 170 g. All together, 124 animals were used. Of these, 58 were kept for the entire observation period under conditions of normal barometric pressure and partial oxygen pressure (Moscow). Thirty-six of the rats lived at high altitudes for one month. Of these, for the first two weeks, 17 were located at Terskol (2000 m above sea level), the next two weeks they were at Ledovaya Baza (4000 m above sea level), and 19 of the rats remained the entire time at Terskol. The other 30 rats were divided into three groups of 10 animals each. They were all subjected to acclimatization to hypoxia in a pressure chamber (Moscow). The animals of the first group were placed into a pressure chamber each day for 6 hours for a period of 2 weeks. The "altitude" on the first day was 3000 m, then it was gradually increased and on the last day was 7000 m. The rats of the second group were placed into a pressure chamber for a period of 3 days continuously. The initial "altitude" in this experiment was also 3000 m and the final, 7000 m. The third group of animals was subjected to the effect of hypoxia for 6 hours a day for a period of 2 weeks, repeatedly three times for a period of 5 months at intervals of 1 month. /199

During the experiment a dynamic observation was made of the condition of the peripheral blood in all the rats. The basic criteria in evaluating the reaction of the blood system to the effect of hypoxia was the amount of hemoglobin, the number of erythrocytes and reticulocytes.

After completion of the experiments (both high-altitude and pressure-chamber) a part of the animals were killed to study the

medulla ossium. The samples of bone marrow were taken in the following order: in animals who had remained in the mountains, 3 weeks following return to Moscow; in the animals, subjected to hypoxia in the pressure chamber, 3 weeks (first group) and 5 weeks (second and third groups) after the last effect.

The statmokinetic indices (SKI) and the amount of DNA in the cellular nuclei were determined in smears of the medulla ossium, prepared by the usual method. In the first case the smears were dyed following Romanovskiy, in the second, following Feyl'gen.

To determine the SKI (the amount of mitotic cells, blocked in the metaphase by spindle-cell toxins), we used colchicine, injected into the rats 4 hours prior to killing (0.12 mg per 100 g of body weight). The SKI were computed by separate computation of the mitoses per 100 nuclear cells of the erythroblast and myeloid series.

The amount of DNA[1] in the cellular elements of the medulla ossium was determined by the method of cytospectrophotometry. For this we used a "probe" cytospectrophotometer with photoelectric registrations of the absorption [7]. Photometric analysis of the cells was made at a wave length of 546 mμ, with an immersion objective of 90 X, photoocular 20 X, 1.3 numerical aperture and 1μ-diameter photometric scanning field. The amount of DNA in the cells was expressed weight units, picograms [8]. /200

To obtain comparable data in each sample we photometrically measured 100 cells, the nuclei of which had a circular or ellipsoidal shape.

In the medulla ossium of the rats, the erythroblast and the myeloblast elements had either a circular or ellipsoidal shape. The characteristics of the method of cytophotometry do not allow making a fine differentiation of the cells, belonging to the various shoots of the medulla ossium. Therefore, in this case we can speak only about the photometry of the "medulla osseous elements" without taking into account what type they belong to. However, this fact had no special significance for us since the problem of the investigation consisted of evaluating the proliferative ability of the medulla ossium as a whole. For this purpose it was sufficient to represent the characteristics of the DNA distribution in a given set of cells, a part of which possesses the ability of proliferate. By photometrically measuring the cells with circular and ellipsoidal nucleim we could guarantee this condition, since the cells with such nuclei in their greater part are capable of dividing [6].

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[1] To investigate the amount of DNA, we used rats who had not received colchicine.

After determining the amount of DNA in any random set of cells of the tissue under analysis and after constructing a histogram of the distribution of the amount of DNA in the individual cells, we could qualitatively characterize the condition of the proliferative processes in this tissue [1, 2, 4, 9].

RESULTS

Before we proceed to a discussion of the materials which pertain directly to the problem of this paper, let us characterize briefly the reaction of the peripheral blood in our experiments. We noted above that the basic criteria of this were the changes in the amount of hemoglobin, the erythrocytes and reticulocytes. These investigations showed that "acclimatization" to hypoxia produced changes in the red blood that are typical of the hypoxic states (increase in the amount of hemoglobin and the number of erythrocytes and reticulocytes) in the four experiments. In one experiment (Terskol) no increase in hemoglobin and erythrocytes was observed. In this case the number of eticulocytes only increased temporarily by the end of the third week residence at high altitude. After one week following return of the animals to Moscow, the number of reticulocytes dropped to the original values. The "traces" of the reaction of the red blood in the other cases were traced for a period of 1 - 2 weeks. This fact in essence determined the time of the investigations of the medulla ossium, the samples of which were taken 3 - 5 weeks following the end of the experiments. /201

From the data given in Table 1, it is obvious that in the experimental rats, regardless of the fact of whether it was high-altitude or pressure-chamber "acclimatization" to which they were subjected, the average value of the statmokinetic indices (SKI) of the erythroblast elements was found to be significantly higher than in the control group. Here we see an approximately equal degree of increase in the mitoses in the cells of a given series: literally in all cases the SKI of the erythroblasts in the experimental rate exceeded their value in the control animals by 50%. Statistical analysis confirmed the reliability of this difference. The SKI of the myeloidal cells, as is obvious from Table 1, inthe rats subjected to the high-altitude acclimatization and in the control rats, were practically the same. After the pressure-chamber effects the value of the statmikinetic indices of the myeloidal cells decreased substantially. Statistical analysis indicates that this decrease is reliable. The obtained data permit the conclusion that acclimatization to hypoxia stimulates the proliferative activity of the erythroblast elements and suppresses the myeloidal elements. Moreover, they indicate the prolonged character of this reaction, noted for a period of 3 - 5 weeks after the end of the experiments and apparently do not depend (at least in the case of the erythroblasts) on the schedule of this latter. It should also be emphasized that the changes in the proliferative activity of the medulla osseous cells observed after acclimatization are retained for a considerably longer period of time than the corresponding changes in the composition of the red blood. They appear even when

the picture of the blood which is typical of the hypoxic conditions is not evolved.

This conclusion agrees fully with the results of determining the DNA in the medulla osseous cells (Table 2). The cytophotometric investigation showed that the amount of DNA in them varies within ω range corresponding to the mitotically multiplying cells: from 6 ± 1 to 12 ± 2 ng, i.e., from a diploidal to a tetraploidal amount The values between these amounts (7.1 - 9.9 ng) may be assumed as belonging to the DNA-synthesizing cells.

Analysis of the variation curves of the amount of DNA in the individual cells of the medulla ossiom of the control rats showed that in these latter animals the number of diploidal cells is on the average 29%, the number of DNA-synthesizing cells is 33%, the number of tetraploidal cells, i.e., the cells completing the DNA synthesis and consequently prepared for future mitosis, 38%. Thus, the total amount of DNA-synthesizing cells and those having completed synthesis in these animals was 71%.

In the rats subjected to altitudes of 2000 and 4000 m, the total number of such cells after 3 weeks following return to Moscow was found to be higher, 80 and 79%, respectively. These data permit

TABLE 1. STATMOKINETIC INDICES (SKI) OF ERYTHROBLASTS AND MYELOIDAL CELLS IN THE MEDULLA OSSIUM AFTER HIGH-ALTITUDE AND PRESSURE-CHAMBER ACCLIMATIZATION. /202

Group	No. of Rats	Time of Investigation of Medulla Ossium After Experiment, weeks	SKI(M+m)	
			Erythroblasts	Myeloidal
Control	35	—	17,0±0,5	6,0±0,42
Terskol (2000m)	4	3	25,5±1,5 P<0,01	6,5±1,7 0,8>P>0,7
Terskol - Ledovaya Baza (2000-4000 m)	4	3	25,0±1,3 P<0,01	5,4±1,4 0,7>P>0,6
Pressure Chamber (3000-70000 m)				
First	10	3	24,0±1,3 P<0,01	4,4±0,17 P<0,01
Second	10	5	24,0±1,1 P<0,01	4,3±0,18 P<0,01
Third	10	5	24,3±1,2 P<0,01	3,2±0,11 P<0,01

TABLE 2. AVERAGE AMOUNT AND DISTRIBUTION OF DNA IN THE CELLS OF THE MEDULLA OSSIUM OF CONTROL AND EXPERIMENTAL RATS.

Group	Number of Animals	Total no. of Investigated Cells	Average Amount of DNA in the nucleus ng	Cells with DNA, %		
				2x (6±1), ng	2x-4x (7.1-9.9), ng	4x (12±2), ng
Control	13	1700	9.6	29	33	38
Terskol (2000 m)	8	800	10.4	20	36	44
Terskol-Ledovaya Baza (2000-4000 m)	7	700	10.1	21	36	43

the conclusion that prolonged residence of the animals in an atmosphere with a lowered amount of oxygen stimulates the synthetic processes which are the basis of preparation of the cells for dividing, and in the same manner facilitates more intense course of the processes of proliferation in the tissue being analyzed. Along with this the obtained results show that this amplification of the biosynthetic processes is retained for along period of time, being a sign of the difficult reversibility of the reaction developed in the medulla ossium. /203

CONCLUSION

Thus the group of data presented showed that residence of animals in an atmosphere with a lowered amount of oxygen was accompanied by the occurrence of definite changes from the side of the peripheral blood, which are typical for hypoxic conditions. With return to normal conditions the qualitative and quantitative composition of the peripheral blood was rapidly normalized. After 1-2 weeks the blood of the animals, subjected to acclimatization, did not differ from the blood of the control rats. However, a pronounced regeneration of the process of cellular reproductibility in the medulla ossium of the rats adapted to hypoxia was retained considerably longer (more than 3 - 5 weeks). This is indicated by the large percent of synthesizing DNA cells and those having completed synthesis, and also by the large amount of mitoses of the erythroblast series. Since no increase in the mitoses of the myloidal cells in the medulla ossium of the experimental animals was noted (the number of these latter, on the contrary, decreased), we may assume that the increase in the number of DNA-synthesizing cells and those having completed synthesis was caused by the stimulation of synthetic processes only in the erythroblast elements. No influence of the various schedules of adaptation on the rate of proliferation of the erythroblast cells was detected. In this respect it is of interest to look at the myeloidal shoots of the medulla ossium; the changes in the proliferative

activity of the myeloidal cells permit tracing not only the influence of the various schedules of acclimatization, but apparently also the possibility of cumulation of changes with the repeated effect of hypoxia on the organism.

Summing up, we can conclude that the amplification of the synthetic processes which are the basis of readying the cells for division, just as the smplification of proliferation of the cells of the erythroblast series, retained for such a long period of time after acclimatization ot hypoxia, is a sign of the difficult reversibility of the changes which occur under the effect of this factor. Analogously, the changes in the myeloidal cells must obviously be evaluated.

REFERENCES

1. Brodskiy, V.Ya: Trofika kletki. (Cell Trophics)., Moscow, 1966.
2. Vladimirskaya, Ye. B., Ye.Ye Simonov, I.S. Balakhovskiy and I. Ye.Ivanova: Problemy genatologii. Vol. 10, No. 9, p. 23, 1965.
3. Voytkevich, V.I.: In the Collection: Materialy nauchnoy konferentsii po problemam fiziologii i patologii kortiko-vistseral'nykh vzaimootnosheniy i funktsional'nykh sistem organizma. (Materials from the Scientific Conference on Problems of Physiology and Pathology of the Corticovisceral Relationships and the Functional Systems of the Organism)., Ivanovo, No. 1, p. 240, 1965. /204
4. Meziya, D.: Mitoz i fiziologiya kletochnogo deleniya. (Mitosis and the Physiology of Cell Division)., Moscow, 1963.
5. Nikolayenko, Yu.V.: In the Collection: Materialy nauchnoy konferentsii po problemam fiziologii i patologii kortiko-vistsral'nykh vzaimootnosheniy i funktsional'nykh sistem organizma. (Materials from the Scientific Conference on Problems of Physiology and Pathology of the Corticovisceral Relationships and the Functional Systems of the Organism)., Ivanovo, No. 2, p. 118, 1965.
6. Sbitneva, M.F., T.V. Kalyayeva and I.A.Rudakov: Byull. eksperim. biol. i med, Vol. 7, No. 5, p. 112, 1964.
7. Simonov, Ye.Ye., and I.S. Balakhovskiy: Laboratornoye delo, No. 3, p. 184, 1965.
8. Simonov, Ye.Ye.: Laboratornoye delo, No. 5, p. 263, 1966.
9. Tsanev, R.G. and G.G. Markov: Biokhimiya kletochnogo deleniya. (Biochemistry of Cell Division), Moscow, 1964.